ATTORNEY'S DOCKET NO.: 2003080-0071 (SK-744-CON4)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Danishefsky *et al.*	Examiner: T. Solola
Serial No.:	not yet assigned	Group Art Unit: 1626
Filed:	December 4, 2001	
For:	*Synthesis of Epothilones, Intermediates Thereto, Analogues and Uses Thereof*	

BOX PATENT APPLICATION
ASSISTANT COMMISSIONER FOR PATENTS
US PATENT AND TRADEMARK OFFICE
P.O. BOX 2327
ARLINGTON, VA 22202

EXPRESS MAIL NO: EL603009481US

Sir:

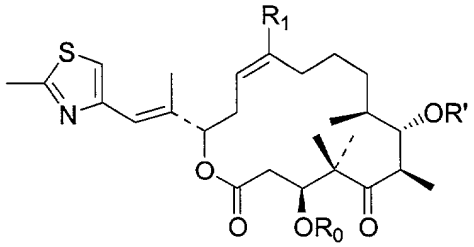
PRELIMINARY AMENDMENT

Applicants respectfully request entry of the following amendments in the continuation application submitted under 37 C.F.R. § 1.53(b) herewith:

In the claims:

Please add claim 59:

59. A compound having the structure:



wherein R_1 is hydrogen or methyl, and R_0 and R' are each hydrogen.

In the specification:

On page 1, starting on line 27 and ending on line 34, please replace the paragraph with the following amended paragraph:

This application is a continuation of and claims priority under 35 U.S.C. § 120 to co-pending application number 09/874,514, filed June 5, 2001, which application is a continuation application of and claims priority under 35 U.S.C. § 120 to 08/986,025, filed December 3, 1997, now U.S. Patent No. 6,242,469, issued June 5, 2001, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial Nos. 60/032,282, 60/033,767, 60/047,941, and 60/055,533, filed December 3, 1996, January 14, 1997, May 22, 1997, May 29, 1997, and August 13, 1997, respectively, the contents of which are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824, CA-39821, CA-GM 72231, CA-62948, and AI0-9355 from the National Institutes of Health, and grant CHE-9504805 from the National Science Foundation. Additionally, the present invention was supported in part by a fellowship from the United States Army to Dongfang Meng (DAMD 17-97-1-7146), and thus the government has certain rights in the invention.

On page 3, lines 21-22, please replace the paragraph with the following amended paragraph:

Figures 3(A) and 3(B) provide syntheses of key iodinated intermediates used to prepare hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives.

On page 3, lines 24-27, please replace the paragraph with the following amended paragraph:

Figures 3(C) and 3(D) provide methods of preparing hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives, said methods being useful generally to prepare 12,13-*E* epothilones wherein R is methyl, ethyl, n-propyl, and n-hexyl from the corresponding *E*-vinyl iodides.

On page 3, lines 29-30, please replace the paragraph with the following amended paragraph:

Figures 3(E) and 3(F) show reactions leading to benzoylated hydroxymethyl-substituted desoxyepothilone and hydroxymethylene-substituted epothilone (epoxide).

On page 4, line 9, please replace the paragraph with the following amended paragraph:

Figures 6(A) and 6(B) provide a scheme of an olefin metathesis route to epothilone A and other analogues.

On page 4, line 29, please replace the paragraph with the following amended paragraph:

Figures 14(A) and 14(B) show the preparation of intermediate **4A**.

On page 5, lines 7-8, please replace the paragraph with the following amended paragraph:

Figures 18(A) and 18(B) provide a synthetic pathway to a protected intermediate for 8-desmethyl deoxyepothilone A.

On page 5, lines 10-11, please replace the paragraph with the following amended paragraph:

Figures 19(A), 19(B) and 19(C) provide a synthetic pathway to 8-desmethyl deoxyepothilone A, and structures of *trans*-8-desmethyl-desoxyepothiolone A and a trans-iodoolefin intermediate thereto.

On page 5, lines 13-22, please replace the paragraph with the following amended paragraph:

Figure 20(A) shows structures of epothilones A and B and 8-desmethylepothilone and Figure 20(B) shows a synthetic pathway to intermediate TBS ester **10** used in the preparation of desmethylepothilone A. (a) (*Z*)-Crotyl-B[(–)-Ipc]$_2$, –78°C, Et_2O, then 3N NaOH, 30% H_2O_2; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (74% for two steps, 87% ee); (c) O_3, CH_2Cl_2/MeOH, –78°C, then DMS, (82%); (d) *t*-butyl isobutyrylacetate, NaH, BuLi, 0°C, then **6** (60%, 10:1); (e)

$Me_4NBH(OAc)_3$, –10°C (50%, 10:1 α/β) or $NaBH_4$, MeOH, THF, 0°C, (88%, 1:1 α/β); (f) TBSOTf, 2,6-lutidine, –40°C, (88%); (g) Dess-Martin periodinane, (90%); (h) $Pd(OH)_2$, H_2, EtOH (96%); (I) DMSO, oxalyl chloride, CH_2Cl_2, –78°C (78%); (j) Methyl triphenylphosphonium bromide, NaHMDS, THF, 0°C (85%); (k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (87%).

On page 5, line 29, please replace the paragraph with the following amended paragraph:

Figures 22(A), 22(B) and 22(C) show a synthetic pathway to prepare epothilone analogue **27D.**

On page 5, line 31, please replace the paragraph with the following amended paragraph:

Figures 23(A), 23(B) and 23(C) show a synthetic pathway to prepare epothilone analogue **24D.**

On page 5, line 33, please replace the paragraph with the following amended paragraph:

Figures 24(A) and 24(B) show a synthetic pathway to prepare epothilone analogue **19D.**

On page 5, line 35, please replace the paragraph with the following amended paragraph:

Figures 25(A), 25(B), 25(C) and 25(D) show a synthetic pathway to prepare epothilone analogue **20D.**

On page 5, line 37, please replace the paragraph with the following amended paragraph:

Figures 26(A), 26(B), 26(C) and 26(D) show a synthetic pathway to prepare epothilone analogue **22D.**

On page 6, lines 1-2, please replace the paragraph with the following amended paragraph:

Figures 27(A), 27(B) and 27(C) show a synthetic pathway to prepare epothilone analogue 12-hydroxy ethyl-epothilone.

On page 6, lines 4-7, please replace the paragraph with the following amended paragraph:

Figures 28(A) and 28(B) show the activity of epothilone analogues in a sedimentation test in comparison with DMSO, epothilone A and/or B. Structures 17-20, 22, and 24-27 are shown in Figures 29-37, respectively. Compounds were added to tubulin (1mg/ml) to a concentration of 10 μM. The quantity of microtubules formed with epothilone A was defined as 100%.

On page 6, lines 30-32, please replace the paragraph with the following amended paragraph:

Figures 39(A) and 39(B) show epothilone A and epothilone analogues #1-7. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

On page 6, lines 34-36, please replace the paragraph with the following amended paragraph:

Figures 40(A) and 40(B) show epothilone B and epothilone analogues #8-16. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

On page 7, lines 1-3, please replace the paragraph with the following amended paragraph:

Figures 41(A) and 41(B) show epothilone analogues #17-25. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

On page 7, lines 5-7, please replace the paragraph with the following amended paragraph:

Figures 42(A) and 42(B) show epothilone analogues #26-34. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

On page 7, lines 10-12, please replace the paragraph with the following amended paragraph:

Figures 42(C) and 42(D) show epothilone analogues #35-46. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

On page 7, line 14, please replace the paragraph with the following amended paragraph:

Figure 42(E) shows epothilone analogues #47-49.

REMARKS

Applicants respectfully request entrance of the amendments as detailed above, in the continuation application filed herewith under 37 C.F.R. § 1.53(b). Applicants respectfully submit that no new matter is presented with these amendments. Rather, the original specification, as filed on December 3, 1997, for the parent application 08/986,025, now issued as U.S. Patent, 6,242,469 has been provided for filing under 37 C.F.R. §1.53 (b), which same specification was provided for filing of co-pending parent application number 09/874,514, filed June 5, 2001. Applicants respectfully submit that this preliminary amendment is requested to correct formal matters in the specification (e.g., addition of a statement of continuation application status (with incorporation by reference), addition of a statement regarding government support, and ensuring consistency between the specification and formal drawings). As required, Applicants have submitted herewith replacement sections and paragraphs for those sections and paragraphs that have been amended as detailed above.

Additionally, Applicants would like to bring a typographical error in the declaration to

the Examiner's attention. Specifically, the name of one inventor Dongfang Meng is incorrectly listed as Dang Fang Meng. Applicants respectfully submit that the <u>correct spelling is **Dongfang Meng**</u>. As set forth in MPEP 605.04(b), "when a typographical or transliteration error in the spelling of an inventor's name is discovered during the pendency of an application, a petition is not required, nor is a new oath or declaration under 37 CFR 1.63 needed". Applicants thus respectfully request that reference is made to this notification on the declaration so that any further correspondence (e.g., filing receipts) and issued patents will reflect the correct spelling of his name.

Applicants would like to thank the Examiner in advance for review of this request. If it is believed that a telephone conversation would expedite matters, the Examiner is invited to contact the undersigned at (617) 248-5216. The Examiner's attention is also directed to the recent change in power of attorney and correspondence address, as submitted herewith. Although it is believed that there is no fee associated with this amendment, if Applicants are mistaken, please charge any fees to our Deposit Account No.: 03-1721.

Respectfully Submitted,

Karoline K. M. Shair, Ph.D.
Reg. No.: 44,332

Choate, Hall & Stewart
Exchange Place
53 State Street
Boston, MA 02109
(617) 248-5216
Date: December 4, 2001
3341729_1.DOC

Marked-Up Copies of Amended Paragraphs

a) Paragraph on page 1, starting on line 27 and ending on line 34:

This application is a continuation of and claims priority under 35 U.S.C. § 120 to co-pending application number 09/874,514, filed June 5, 2001, which application is a continuation application of and claims priority under 35 U.S.C. § 120 to 08/986,025, filed December 3, 1997, now U.S. Patent No. 6,242,469, issued June 5, 2001, which claims priority under 35 U.S.C. § 119(e) to [is based on] U.S. Provisional Application Serial Nos. 60/032,282, 60/033,767, 60/047,941, and 60/055,533, filed December 3, 1996, January 14, 1997, May 22, 1997, May 29, 1997, and August 13, 1997, respectively, the contents of which are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824, CA-39821, CA-GM 72231, CA-62948, and AI0-9355 from the National Institutes of Health, and grant CHE-9504805 from the National Science Foundation. Additionally, the present invention was supported in part by a fellowship from the United States Army to Dongfang Meng (DAMD 17-97-1-7146), and thus the government has certain rights in the invention.

b) Paragraph on page 3, lines 21-22:

[Figure 3A provides] Figures 3(A) and 3(B) provide syntheses of key iodinated intermediates used to prepare hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives.

c) Paragraph on page 3, lines 24-27:

[Figure 3B provides] Figures 3(C) and 3(D) provide methods of preparing hydroxymethylene- and hydroxypropylene-substituted epothilone derivatives, said methods being useful generally to prepare 12,13-*E* epothilones wherein R is methyl, ethyl, n-propyl, and n-hexyl from the corresponding *E*-vinyl iodides.

d) Paragraph on page 3, lines 29-30:

[Figure 3B shows] Figures 3(E) and 3(F) show reactions leading to benzoylated

hydroxymethyl-substituted desoxyepothilone and hydroxymethylene-substituted epothilone (epoxide).

e) Paragraph on page 4, line 9:

[Figure 6 provides] Figures 6(A) and 6(B) provide a scheme of an olefin metathesis route to epothilone A and other analogues.

f) Paragraph on page 4, line 29:

[Figure 14 shows] Figures 14(A) and 14(B) show the preparation of intermediate **4A**.

g) Paragraph on page 5, lines 7-8:

[Figure 18 provides] Figures 18(A) and 18(B) provide a synthetic pathway to a protected intermediate for 8-desmethyl deoxyepothilone A.

h) Paragraph on page 5, lines 10-11:

[Figure 19 provides] Figures 19(A), 19(B) and 19(C) provide a synthetic pathway to 8-desmethyl deoxyepothilone A, and structures of *trans*-8-desmethyl-desoxyepothiolone A and a trans-iodoolefin intermediate thereto.

i) Paragraph on page 5, lines 13-22:

[Figure 20 shows (top)] Figure 20(A) shows structures of epothilones A and B and 8-desmethylepothilone and [bottom] Figure 20(B) shows a synthetic pathway to intermediate TBS ester **10** used in the preparation of desmethylepothilone A. (a) (*Z*)-Crotyl-B[(–)-Ipc]$_2$, –78°C, Et_2O, then 3N NaOH, 30% H_2O_2; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (74% for two steps, 87% ee); (c) O_3, CH_2Cl_2/MeOH, –78°C, then DMS, (82%); (d) *t*-butyl isobutyrylacetate, NaH, BuLi, 0°C, then **6** (60%, 10:1); (e) $Me_4NBH(OAc)_3$, –10°C (50%, 10:1 α/β) or $NaBH_4$, MeOH, THF, 0°C, (88%, 1:1 α/β); (f) TBSOTf, 2,6-lutidine, –40°C, (88%); (g) Dess-Martin periodinane, (90%); (h) $Pd(OH)_2$, H_2, EtOH (96%); (I) DMSO, oxalyl chloride, CH_2Cl_2, –78°C (78%); (j) Methyl triphenylphosphonium bromide, NaHMDS, THF, 0°C (85%); (k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (87%).

j) Paragraph on page 5, line 29:

[Figure 22 shows] Figures 22(A), 22(B) and 22(C) show a synthetic pathway to prepare epothilone analogue **27D.**

k) Paragraph on page 5, line 31:

[Figure 23 shows] Figures 23(A), 23(B) and 23(C) show a synthetic pathway to prepare epothilone analogue **24D.**

l) Paragraph on page 5, line 33:

[Figure 24 shows] Figures 24(A) and 24(B) show a synthetic pathway to prepare epothilone analogue **19D.**

m) Paragraph on page 5, line 35:

[Figure 25 shows] Figures 25(A), 25(B), 25(C) and 25(D) show a synthetic pathway to prepare epothilone analogue **20D.**

n) Paragraph on page 5, line 37:

[Figure 26 shows] Figures 26(A), 26(B), 26(C) and 26(D) show a synthetic pathway to prepare epothilone analogue **22D.**

o) Paragraph on page 6, lines 1-2:

[Figure 27 shows] Figures 27(A), 27(B) and 27(C) show a synthetic pathway to prepare epothilone analogue 12-hydroxy ethyl-epothilone.

p) Paragraph on page 6, lines 4-7:

[Figure 28 shows] Figures 28(A) and 28(B) show the activity of epothilone analogues in a sedimentation test in comparison with DMSO, epothilone A and/or B. Structures 17-20, 22, and 24-27 are shown in Figures 29-37, respectively. Compounds were added to tubulin (1mg/ml) to a concentration of 10 μM. The quantity of microtubules formed with epothilone A was defined as 100%.

q) Paragraph on page 6, lines 30-32:

[Figure 39 shows] Figures 39(A) and 39(B) show epothilone A and epothilone analogues #1-7. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

r) Paragraph on page 6, lines 34-36:

[Figure 40 shows] Figures 40(A) and 40(B) show epothilone B and epothilone analogues #8-16. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

s) Paragraph on page 7, lines 1-3:

[Figure 41 shows] Figures 41(A) and 41(B) show epothilone analogues #17-25. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

t) Paragraph on page 7, lines 5-7:

[Figure 42(A) shows] Figures 42(A) and 42(B) show epothilone analogues #26-34. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

u) Paragraph on page 7, lines 10-12:

[Figure 42(B) shows] Figures 42(C) and 42(D) show epothilone analogues #35-46. Potencies against human leukemia CCRF-CEM (sensitive) and CCRF-CEM/VBL MDR (resistant) sublines are shown in round and square brackets, respectively.

v) Paragraph on page 7, line 14:

[Figure 42(C) shows] Figure 42(E) shows epothilone analogues #47-49.